

5.1.37

AOAC Official Method 959.18 Nitromide in Feeds

Spectrophotometric Method

First Action 1959

Final Action

A. Reagents

(a) *Diethylamine reagent (DEA), aged*.—(One year or older.) Fresh DEA may be artificially aged as follows: Place 1 L DEA in dry 2 L flask with 40 g sodium or potassium fluosilicate. Connect flask to 60 cm (24 in.) bulb reflux condenser and reflux on sand bath 2–3 days in hood. When reagent is sufficiently “aged,” 2 mL clear DEA added to 8 mL dimethylsulfoxide containing 50 g 3,5-DNBA should develop maximum color in ca 40 min. *A* as read on Beckman DU spectrophotometer at 560 nm should be ca 0.375; on Klett-Summerson photoelectric colorimeter with No. 56 filter, ca 200. Reagent must be free from turbidity. Prepare new standard curve for each batch of DEA.

(b) *Nitromide standard solutions*.—(1) *Stock solution*.—1 mg/mL. Weigh 100 mg 3,5-dinitrobenzamide into 100 mL flask and dilute to volume with methanol. (2) *Working solution*.—20 g/mL. Transfer 2.0 mL stock solution to 100 mL volumetric flask and dilute to volume with methanol.

B. Preparation of Standard Curve

Place 1.0, 2.0, 3.0, and 5.0 mL working solution containing 20, 40, 60, and 100 g, respectively, of nitromide in 4 colorimeter tubes. Evaporate to dryness at 50 C in air current. Dissolve residue in 8 mL dimethylsulfoxide at 70 C, cool, and add 2 mL DEA reagent. Place

in dark at 20 –25 C and read after 1 h. Plot standard curve, using *A* as ordinate and concentration as abscissa.

C. Extraction

Weigh 5.0 g test portion of ground feed, containing 250 mg/kg nitromide, into 100 mL volumetric flask and dilute to volume with methanol. Shake frequently 20 min and let stand 40 min to permit feed particles to settle.

If feed contains 750 mg/kg nitromide, use 2 g finely ground test portion; if 1500 mg/kg, use 1 g in 100 mL or 5 g in 500 mL methanol. Prepare premixes by weighing appropriate test portion and serially diluting methanol extract.

D. Determination

Pipet 4 mL aliquot of extract into glass-stoppered test tube. Place tube in 50 C water bath and evaporate to dryness with air current directed onto surface of methanol. Add 8 mL dimethylsulfoxide and heat to 70 C to hasten solution; cool, and add 2 mL DEA reagent. Place in dark 1 h at 20 –25 C. Determine *A* at 560 nm in Beckman DU spectrophotometer, Klett-Summerson photoelectric colorimeter with No. 56 filter, or similar instrument, against dimethylsulfoxide as reference.

Determine amount of nitromide in tube from standard curve.

$$\text{Nitromide in feed, \%} = \frac{\text{g nitromide in tube}}{25} \times \frac{100}{5000000}$$

or $\text{g nitromide in tube} \times 5 = \text{mg nitromide/kg feed (ppm)}$.

Reference: *JAOAC* **42**, 239(1959).

CAS-121-81-3 (nitromide)